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The Relation of Temperature to the Effect of Hydrogen- and Hydroxyl-ion Concentration on *Sclerotinia Fructicola* and *Fomes Annosus*. Spore Germination and Growth

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PAUL E. TILFORD

The growth of fungi is greatly influenced by environment. A certain combination of environmental factors may favor a particular fungus to the extent that it grows rapidly and is a virulent parasite; whereas under another set of conditions the same organism may be weakly parasitic, grow saprophytically, or remain entirely dormant. Considerable is known relative to the influence of separate environmental factors on the activities of many species of fungi, but the relation of one factor to the effect of another in the environmental complex has not been adequately studied.

Temperature and the hydrogen-ion concentration of the medium are factors which exert separately a great influence on the growth rate of mycelium and the germination of spores. The relation of temperature, however, to the effect of pH on these fungous activities has not been thoroughly investigated and such questions as the following are for the most part unanswered: What will be the result of a gradation of temperature, from the lower to the upper temperature limits for growth or for spore germination, on the optimum pH of the medium for growth or for germination? Will the range of pH of the medium for germination of spores or for growth of a fungus be the same at all temperatures at which germination or growth will occur? If a reaction of the medium is slightly retarding in its effect at one temperature, will the same reaction retard growth at all temperatures throughout the temperature range for growth?

The present paper gives the results obtained from a study of the relation of temperature to the effect of hydrogen- and hydroxyl-ion concentration on (a) the growth of *Sclerotinia fructicola* (Wint.) Rehm and *Fomes annosus* (Fr.) Cke. and (b) conidiospore germination of the first-mentioned fungus.

PREVIOUS RELATED WORK

Brooks (2) was the first to study the relation of temperature to the toxicity of acids. Solutions of nitric acid, sulfuric acid, and copper sulfate in sugar beet decoction were used as media for the germination of the spores of several fungi. In all cases the toxic effect of the acids, as well as that of copper sulfate, was least at the optimum temperature for the particular organism. Spores kept in contact with a toxic agent at a temperature that would allow no germination germinated when brought to a favorable temperature and placed in a non-toxic medium. Dry-weight determinations of the total growth produced in liquid cultures confirmed the results obtained by germinating spores in that the toxic agents were least injurious at temperatures near the optimum.

[1]Submitted to the Graduate School of the University of Wisconsin as a thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Our present-day method of expressing hydrogen-ion concentration was not in use at the time Brooks did his work. Acid concentrations expressed in normality indicate total acidity rather than the amount of hydrogen ions. Later discussion of the present work in which the concentration of hydrogen ions is expressed in terms of pH will show that the results confirm Brooks' conclusion in that the hydrogen and hydroxyl ions are least toxic at temperatures near the optimum for the particular fungus.

Itano and Neil (12), in considering the influence of temperature and hydrogen-ion concentration upon the spore cycle of *Bacillus subtilus*, found that spore germination did not occur at 5° C. at any hydrogen-ion concentration but that at 25° and 37°, if the reaction of the broth was between pH 5 and pH 10, the spores germinated. Completion of the spore cycle likewise required a similar hydrogen-ion concentration.

The relation of temperature and hydrogen-ion concentration to urediniospore germination of two biologic forms of *Puccinia graminis tritici* was studied by Hursch (11). His curves show that the pH range over which germination occurs tends to narrow at the higher temperatures.

Johnson (13) worked with several soil molds and found that lowering the temperature from 23° to 10° or raising it from 23° to 33° C. greatly altered the pH limits of growth for *Mucor glomerula* and *Fusarium oxysporum*. Growth occurred at lower pH values at 23° than at either of the other two temperatures.

Eight wood-destroying fungi were grown in three different media adjusted to different hydrogen-ion concentrations and at 15°, 25°, and 35° C. by Wolpert (30). The widest optimum pH ranges were obtained in the most favorable medium, peptone solution. The optimum range varied slightly with the temperature, and a temperature too high or too low for optimum growth tended to narrow and sometimes to shift the optimum pH range.

Webb and Fellows (27) grew *Ophiobolus graminis* in a variety of media of different pH levels and at temperatures of 16°, 20°, and 28° C. They found the optimum reaction for fungous growth to be influenced by the temperature, but temperature did not materially influence the pH range through which growth occurred. Neither was the optimum temperature influenced by variations in the hydrogen-ion concentration.

SPORE GERMINATION

MATERIALS AND METHODS

A modified form of the Van Tieghem cell similar to that described by Duggar (7) was employed. Petri dishes (15 x 90 millimeters) with ground glass covers were obtained, and glass rings supporting cover slips were simply set in these dishes.

A nutrient mannite solution similar to the one used by Webb (25) was used in the present work. Stock solutions of M/5 mannite in N/10 H_3PO_4 and M/5 mannite in N/5 NaOH were prepared and sterilized. The media of different hydrogen-ion concentrations for the germination tests were made by mixing the two stock solutions in varying amounts, as indicated by the titration curve of phosphoric acid given by Clark and Lubs (3), to give the desired pH. The reaction was checked colorimetrically.

The spore suspension was prepared by pouring about 5 cubic centimeters of the medium of the desired pH on a one-week-old potato dextrose agar slant culture of *Sclerotinia fructicola*. The tube was then shaken vigorously, and, if the spore population of the suspension was not dense enough, it was transferred to a second slant culture and shaken. A suspension having about 25 spores to a single low-power field of the microscope was thought about right. A drop of the suspension was transferred to a sterile cover slip which was then inverted and placed on a ring in one of the petri dishes containing about 10 cubic centimeters of the nutrient solution of the same pH as the spore suspension. Triplicate cultures were set up in each dish. The cultures were incubated at constant temperatures of 8°, 13°, 17°, 21°, 25°, 29°, 33°, and 40° C.

Counts were made after 24 hours. From 50 to 100 spores were counted in each of the triplicate cultures. The presence of a germ tube was taken as the criterion of germination. Spores which were only swollen were not considered germinated. The percentage of spores germinated at any given temperature and pH was determined by averaging the results of the three cultures or, in other words, from observing from 150 to 300 spores.

EXPERIMENTAL RESULTS

The spore germination data obtained at the various temperatures and hydrogen-ion concentrations are shown graphically in Figure 1. It is possible

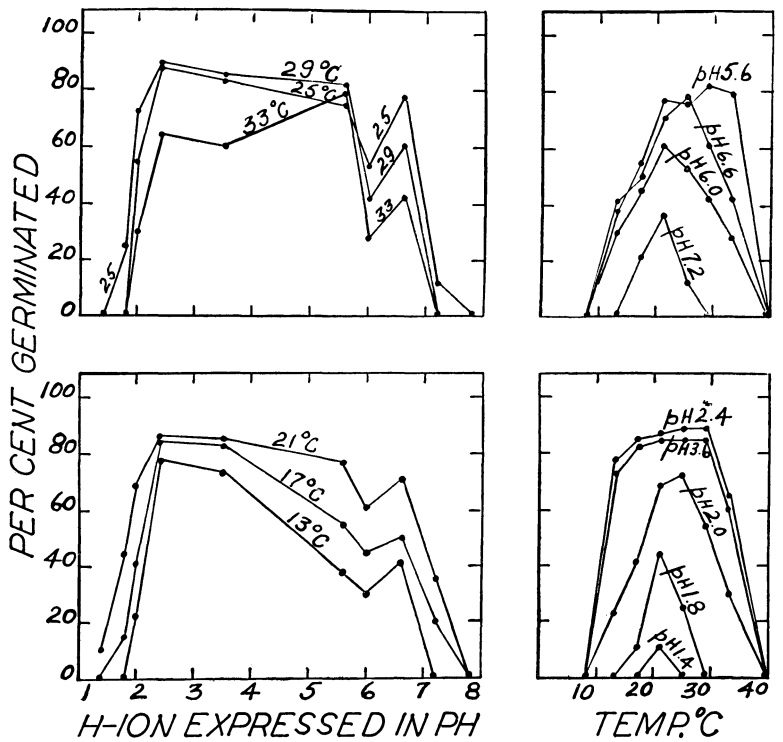

Fig. 1.—The effect of pH and temperature on the germination of *Sclerotinia fructicola* conidiospores

that more spores would have germinated if the time period had been longer than 24 hours, but complications arise if the time is extended to 48 hours. In cultures where there is little or no retardation, after 48 hours mycelial development has progressed so far that it is impossible to make an accurate count.

Temperature and pH limits.—The effect of hydrogen-ion concentration on the germination of the spores of *Sclerotinia fructicola* in mannite solutions is intimately related to the temperature, with respect to the limits of pH for germination. In these experiments the pH limits extended from 1.4 to 7.2 at 21° C. and from 1.8 to 7.2 at 17° and 25°; whereas at 13°, 29°, and 33° germination occurred only from pH 2.0 to 6.6. It is evident that the widest pH range for germination is at the most favorable temperatures, and, as the temperature becomes unfavorable (either up or down), the pH range allowing germination becomes narrower. In this respect the results are similar to those of Brooks (2), in that he found that the harmful effects of a toxic agent were least at the optimum temperature.

The temperature limits for germination are likewise influenced to some extent by the hydrogen-ion concentration. For instance, the spores of *Sclerotinia fructicola* germinated at temperatures ranging from 17° to 25° C. at a reaction of pH 1.8; at reactions of 2.0 to 6.6 germination occurred at temperatures from 13° to 33°; but at pH 7.2 the range was restricted to from 17° to 25°. No germination occurred at any reaction when the temperature was either 8° or 40°. Perhaps, if the time limit had been longer some spores would have germinated at 8°.

Optimum reaction.—The optimum reaction, or the reaction of the medium allowing the highest percentage of spores to germinate, was fairly constant and not influenced by the temperature except in one instance. Germination was better at pH 2.4 at all temperatures tried, except 33° C., than at any other reaction. At 33° C. the best germination was at pH 5.6. Webb's (25) results give an indication of this, in that a temperature change of 4° or 5° C. on either side of the optimum did not materially influence the germination of fungous spores as related to hydrogen-ion concentration.

Optimum temperature.—If the optimum temperature for germination is taken to be the temperature at which the highest percentage of spores germinates in a given time, it is not constant but varies with the reaction of the medium. The best germination occurred at 21° C. when the reaction was pH 2.0 and 6.6 and at 29° when the reaction was 2.4 and 5.6. At pH 3.6 the optimum temperature extended from 21° to 29° C.

Isoelectric point.—Proteins are amphoteric in nature and dissociate either as acids or bases depending on the electrical charge of their environment, as determined by the hydrogen-ion concentration. When a protein is subjected to a range of reactions, a pH can be found where ionization is at a minimum, and this particular pH is called the isoelectric or "neutral" point of the protein. The most satisfactory explanation of double maxima curves obtained when the relation of hydrogen-ion concentration to phenomena, such as spore germination and mycelial growth, is studied has been on the basis of the isoelectric point. The isoelectric point of a fungus, then, occurs at the particular pH where the activity of the protoplasm is at a minimum. It is more or less the resultant of the different isoelectric points of the various ampholytes making up the fungous structure.

A distinct minimum occurs at pH 6.0 in the germination curves of *Sclerotinia fructicola* at all of the temperatures (see Fig. 1), indicating an isoelectric point in this region. Robbins (18) reported an isoelectric point at pH 5.0 to 5.2

for *Rhizopus nigricans*. There is a tendency toward a secondary maxima in the germination curves published by Webb (26), especially in the work with *Botrytis cinerea*. Hopkins (10), Scott (19, 20), and Lindfors (15) have each reported double maxima curves when the effect of hydrogen-ion concentration on fungous growth was considered.

GROWTH STUDIES

MATERIALS AND METHODS

Two solid media were used in the growth studies—malt agar and potato dextrose agar. The malt agar was prepared according to the following formula: 15 grams of Bacto agar, 25 grams of malt extract (Trommer's), and distilled water to make 1000 cubic centimeters. The potato dextrose medium contained the following ingredients: broth from 200 grams of peeled potatoes, 20 grams of dextrose, 20 grams of Bacto agar, and distilled water to make 1000 cubic centimeters. After the flasks of media were autoclaved, the necessary amount of sterilized chemicals was added to each flask to give the desired pH. The contents were well shaken and petri plates poured, each containing about 20 cubic centimeters of the medium. The dishes with ground glass covers were also used for this work.

Varying amounts of sterile M/3 H_3PO_4, NaH_2PO_4, Na_2HPO_4, and Na_3PO_4, as indicated in Table 1, were added to the melted potato dextrose agar after sterilization. The malt agar was adjusted in the same manner, except that the small amount of H_2SO_4 was not needed to get a satisfactory range of pH. The reactions obtained were not the same for malt agar as for potato dextrose but the method gave a satisfactory pH range in each case. The only variable introduced was the sodium-ion concentration, since the phosphate-ion and the dilution were the same. Sodium is a relatively non-toxic ion in such small amounts when it is properly antagonized, as it is in such media as the above.

Electrometric methods which have been described elsewhere by the author (23) were used in determining the pH of all solid media.

TABLE 1.—The Amount of Phosphates Necessary to Adjust the Reaction When Added to 1 Liter of Sterile Potato Dextrose Agar, to Which 0.54 Cubic Centimeter of Concentrated H_2SO_4 has been Added

Approximate pH	Amount of M/3 phosphates to add to 1000 cc. of medium			
	H_3PO_4	NaH_2PO_4	Na_2HPO_4	Na_3PO_4
	Cc.	*Cc.*	*Cc.*	*Cc.*
2.00	100.0	.....	.....	.....
2.25	48.0	52.0	.....	.....
2.35	32.0	68.0	.....	.....
2.50	16.5	83.5	.....	.....
2.60	.....	99.0	1.0	.....
2.85	.....	83.0	17.0	.....
3.25	.....	60.0	40.0	.....
4.60	.....	30.0	70.0	.....
5.75	.....	10.0	90.0	.....
6.20	.....	.....	93.0	7.0
6.35	.....	.....	87.0	13.0
6.80	.....	.....	60.0	40.0
7.15	.....	.....	30.0	70.0
7.45	.....	.....	10.0	90.0

Flecks of mycelium transferred from a vigorously growing culture to the center of the medium in the petri dishes served as inoculum. After inoculation, the plates were placed in waxed paper bags to prevent rapid loss of moisture and incubated at constant temperatures of 5°, 10°, 15°, 20°, 25°, 30°, 35°, and 40° C. It is not thought that the temperature differences altered the pH of the buffered media, since Walbum (24) has shown that the effect of temperature on the reaction of phosphate buffer mixtures is negligible.

Two measurements, at right angles to each other, were made twice daily of the diameters of the mycelial disks after the lag phase had passed. The measurements were taken until the colonies of *Sclerotinia* and *Fomes* reached diameters of 30 and 50 millimeters, respectively, or for one week in case the indicated diameters were not reached. Duplicate plates were used at each temperature and at each pH and the readings were averaged.

EXPERIMENTAL RESULTS

A preliminary experiment was thought advisable before the growth studies were undertaken to determine if any change would occur in the reaction of agar media, buffered with phosphates, upon incubation for 7 days at the temperatures to be used in the experiments. Potato dextrose agars with initial pH values of 2.04, 4.36, 6.14, and 7.72 were prepared. Plates were poured and duplicate plates at each reaction were placed in each of the incubators in the same manner that the cultures were to be treated later.

After 7 days the plates were removed and the reaction in each determined. In most instances the final reaction did not differ from the original by over 0.05 pH, and it was neither consistently higher nor lower than the original. In only four cases was the difference between the original and final reaction as much as 0.1 pH. These small changes in pH are thought to be due to experimental error and are not considered significant. Any change in the reaction of the media in the following experiments is considered due to the action of the fungus.

The average diameters in millimeters of the mycelial disks of *Sclerotinia fructicola* grown on malt agar and on potato dextrose agar, adjusted to various pH levels and held at different constant temperatures, are given in Appendices I and II. Similar data for *Fomes annosus* are included in Appendices III and IV. Figures 2, 3, 4, and 5 are graphical presentations of the data. Measurements were made until the cultures of *Sclerotinia* and *Fomes* reached average diameters of 30 and 50 millimeters, respectively, or for 7 days if the cultures did not reach the stated diameters. The data were taken in this fashion so that they could be analyzed on the basis of the number of hours required to do a given amount of work. Seven days were set as the limit of the experiments because it was thought that further readings would be unreliable, since the organisms altered the hydrogen-ion concentration of the media considerably.

Both organisms produced large amounts of acid and altered the pH of the media, especially when growing at reactions above the optimum for the particular fungus. The change in reaction was not very great when *Sclerotinia fructicola* was grown on either malt or potato dextrose agars below an initial reaction of pH 4. Increasingly large amounts of acid were produced, however, as the initial pH was raised above 4; for instance, the final reaction of potato dextrose agar of initial pH 6.99 was 4.80 after the fungus had produced 33 millimeters of radial growth at 30° C. The final reaction was 2.19 pH units more acid than at the beginning. Since no change in the reaction of the media

occurred in sterile plates, in this particular case, then, the organism produced enough acid to increase the hydrogen-ion concentration of the medium by over 100 fold. *Fomes annosus* produced only small amounts of acid when the initial reaction was below pH 5. The final reaction of media of an initial pH of 7 or above was usually lower by at least 2 pH. Most investigators—Cummins (6), Dunn (8), Klotz (14), MacInnes (16), Scott (19), Wolpert (30), Weimer and Harter (29), and Young and Bennett (31)—who have studied the effect of fungous growth on the reaction of culture media have found that if abundant carbohydrate is present a large amount of acid is produced. Wehmer (28), in an extensive series of studies, found that fungi under certain environmental conditions produce large amounts of oxalic acid. He reported little or no acid formation when abundant organic free acids were present in the medium, but oxalic acid was formed in large amounts in the presence of basic phosphates. According to Cooley (5) *Sclerotinia cinerea* produces oxalic acid when grown on either a fruit juice medium or on peaches.

The fact that the media, even though buffered, do not remain at a constant pH greatly complicates a study of this kind. It should be borne in mind that throughout the present discussion when a culture at a certain pH is mentioned the initial reaction is referred to.

Shape of the curves.—It is generally accepted that the rate of mycelial extension of a fungus, in a strictly vegetative condition in culture under constant environment, is very slow at first but increases rapidly until a maximum rate is reached. Extension then continues at this rate, which is proportional to time, until the environment has changed because of the activities of the fungus to the extent that the growth rate is retarded. Consequently, a typical curve for mycelial extension plotted against time is convex at first but rapidly approaches a straight line and continues as a straight line until, of course, it becomes concave when retardation occurs. The period of slow growth with increasing growth rate at the start is called the lag phase and the length of time that any particular culture is in the lag phase depends on the organism, the size of inoculum, type of media, and probably the congeniality of all of the other environmental factors. In most instances in the literature, the growth measurements of mycelial extension have not been made at short enough intervals to emphasize the convex part of the curve, and, as a result, straight lines are obtained. Bateman (1) found the rate of extension of *Fomes annosus* mycelium to be directly proportional to time, or, in other words, a straight-line relationship.

The curves in the present work, shown in Figures 2, 3, 4, and 5, with a few exceptions, are straight lines. The measurements were not taken early or often enough during the lag phase to emphasize its influence in the curves. There was a tendency for the growth rate of cultures at initial hydrogen-ion concentrations slightly above the optimum to increase with time, especially at favorable temperatures. This is illustrated by the curve for pH 6.80 in Figure 2 at temperatures 20° and 25° C., the curve for pH 6.99 in Figure 3 at 30°, and the curve for pH 7.13 at 20°, 25°, 30°, and 35° in Figure 5. Both of the fungi caused a great decrease in the pH of the media, especially when the initial reaction was above the optimum range. Undoubtedly, the increase in the growth rate of the particular cultures just mentioned can be explained by the fact that as growth occurred acid was produced and the reaction became more favorable for growth.

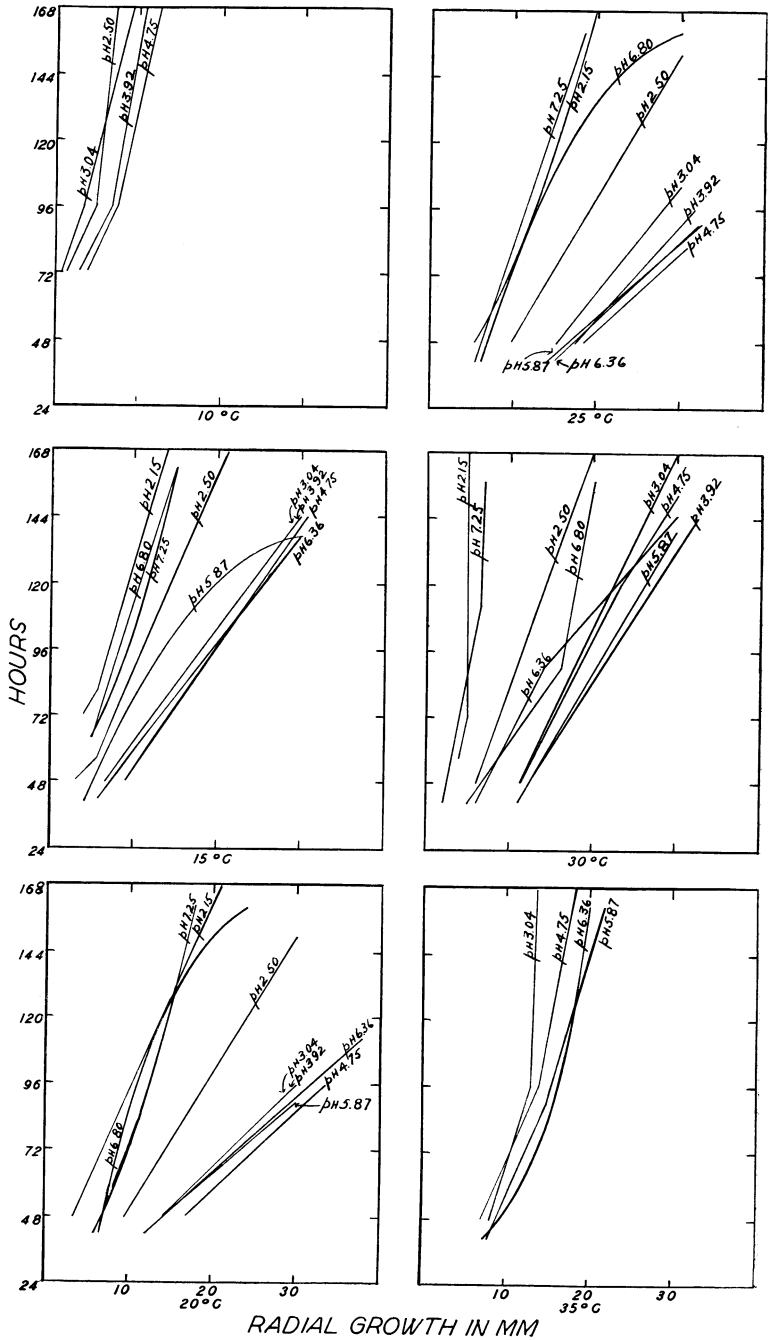

Fig. 2.—The effect of pH and temperature on the growth rate of *Sclerotinia fructicola* on malt agar

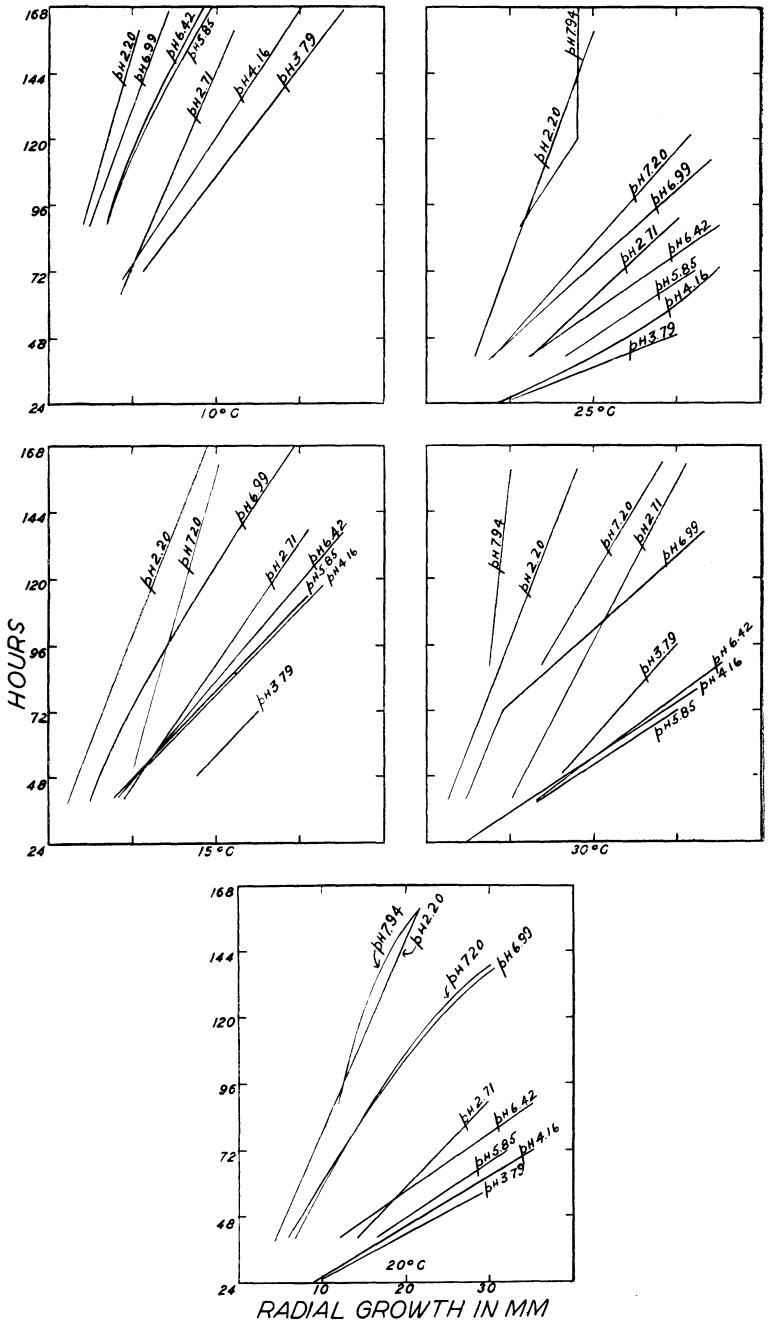

Fig. 3.—The effect of pH and temperature on the growth rate of *Sclerotinia fructicola* on potato dextrose agar

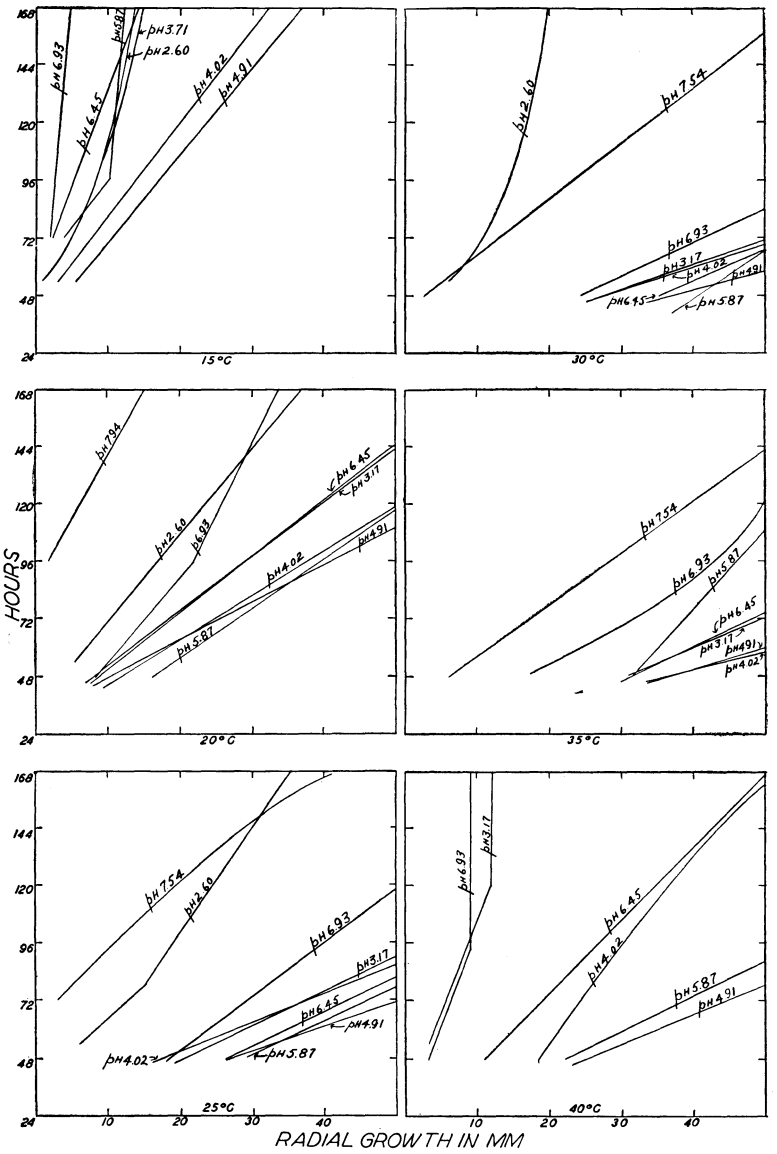

Fig. 4.—The effect of pH and temperature on the growth rate of *Fomes annosus* on malt agar

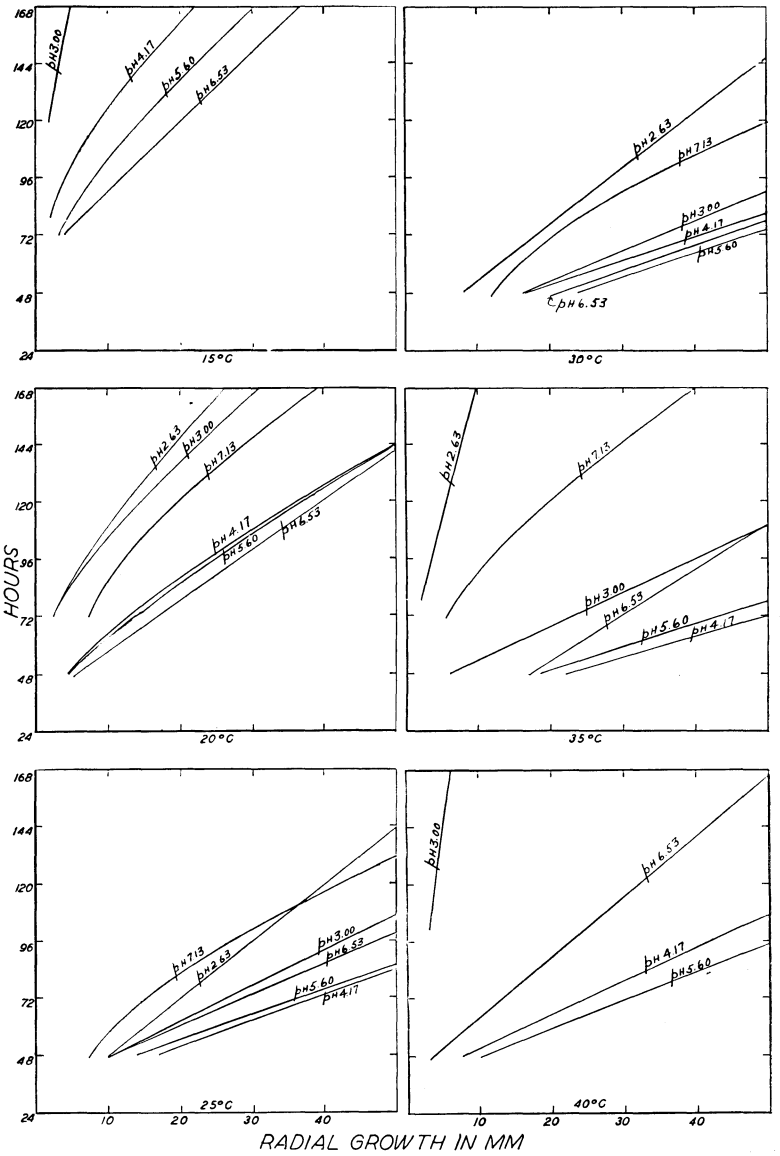

Fig. 5.—The effect of pH and temperature on the growth rate of *Fomes annosus* on potato dextrose agar

Certain of the curves on the acid side of the reaction range show that an increase in growth rate occurred—for example, the curves for *Fomes annosus* on potato dextrose agar at pH 2.63 and 3.00 at 20° (Fig. 5). These reactions are below the optimum reaction range and at a temperature below the optimum. Under these conditions the lag phase would be prolonged; evidently, these cultures were still in this phase, with increasing growth rate, when the first measurements were made. The increase in growth rate in these instances can not be explained by assuming that the reaction became more favorable with extended growth since the pH remained almost constant.

There is still another type of variation from straight lines, which is well illustrated by the curves for *Sclerotinia fructicola* on malt agar in Figure 2 at temperatures of 10° and 35° C. Cultures which were grown at near the temperature limits were likely to stop growing suddenly or continue at a much reduced rate. In some instances, this occurred in cultures growing at a favorable temperature but on media whose hydrogen-ion concentration was near the limits for growth. The curves for pH 2.60 at 25° and 30° C. in Figure 4 and for pH 7.94 at 25° in Figure 3 show this type of deviation from straight lines.

Cummins (6) was of the opinion that the rate of growth of *Fomes annosus* on malt agar was not proportional to the time. He states that: "The rate of growth increases during the early stages of growth and appears to tend towards a maximum rate which to the limits of the experiments is roughly proportional to the time". This is just another way of stating that after the lag phase the growth is proportional to time. Nothing can be found in the present work to indicate that the growth rate of either *Sclerotinia fructicola* or *Fomes annosus* is not proportional to the time to the end of the experiments, after the lag phase is past, except in the few cases which have been mentioned.

Toxicity of hydrogen and hydroxyl ions.—The present work bears out the generally accepted conclusion that the hydrogen ion is much less toxic to fungi than the hydroxyl ion. Growth in all cases occurred far down on the acid side of neutrality, but very little growth took place in the case of either of the organisms at reactions much above neutrality.

There is an indication that the hydrogen ion is more toxic at the upper temperature limits for growth than at the lower limits. The hydroxyl ion, on the other hand, apparently tends to be more toxic at the lower limits of temperature for growth. *Sclerotinia fructicola* on malt agar produced some growth when the initial reaction was pH 2.50 at 10° C.; whereas the culture at this reaction at 35° did not grow. When the initial reaction was pH 5.87 and 6.36, fair growth occurred at 35° C. but very little at 10° C. The data for *Fomes annosus* show the same relationships. On malt agar, for instance, this organism grew slowly at pH 2.60 at 15° C., but no growth was produced at 40° C.; whereas at pH 5.87 and 6.45 good growth occurred at 40°, but at 15° it was very poor. The same fungus on potato dextrose agar at pH 2.63 did not grow at either 15° or 40° C., and the growth at pH 3.00 was about the same at the two temperatures. Growth proceeded at a much faster rate at pH 5.60 and 6.53 at 40° C., however, than at 15°.

Effect of temperature on the pH limits for growth.—The exact limits of hydrogen-ion concentration which permit growth were not determined. In each case some growth occurred at some temperature on the most acid and the most alkaline media tried. The data do, however, indicate that the pH limits for growth depend on the temperature. *Sclerotinia fructicola* on malt agar grew at 10°, 15°, 20°, 25°, and 30° C. when the initial reaction of the media ranged from pH 2.15 to 7.25; whereas at 35° growth occurred only between

3.04 and 6.80. On potato dextrose agar the same organism grew at 10° C. when the initial reaction extended from pH 2.2 to 6.99, and at 15°, 20°, 25°, and 30° from 2.2 to 7.94. At 15° C., *Fomes annosus* grew when the initial reaction of potato dextrose agar was from pH 3.00 to 6.53; whereas at 20°, 25°, 30°, and 35° the range extended from 2.63 to 7.13 and narrowed down again at 40° to from 3.00 to 6.53. When grown on malt agar, *Fomes annosus* grew at initial pH values from 2.60 to 6.93 at 15° C.; from 2.60 to 7.54 at 20°, 25°, and 30°; from 3.17 to 7.54 at 35°; and from 3.17 to 6.93 at 40°.

The effect of temperature on the pH limits for growth of *Sclerotinia fructicola* and *Fomes annosus* can be summarized by stating that when the temperature is favorable for growth the limits are extended and growth occurs over the maximum range but when the temperature is decidedly unfavorable in either direction the pH range permitting growth is narrowed. The same relationship holds with respect to the temperature limits; when the reaction of the medium is favorable growth occurs over a wide temperature range, other factors being constant, but if the reaction is unfavorable the temperature range is contracted.

Efficiency in growth.—The influence of temperature and of the pH of the medium on the rate of growth of the two fungi is shown graphically in Figures 2, 3, 4, and 5. When the diameters of the colonies after different periods of time are plotted as abscissae and the time as ordinates, as they have been in the present work, the rate of growth is indicated by the slope of the curves. The curves for relatively rapidly growing cultures are more nearly horizontal than those of the slower growing cultures. In Figure 3, for example, the curves for the cultures of *Sclerotinia fructicola* grown on potato dextrose agar at 10° C. show by their slope with respect to the horizontal axis that growth was the most rapid at pH 3.79 and slowest at 2.2. At 15° C. the slope of all of the curves is less than at 10°, indicating that growth was more rapid at the higher temperature. The rate of growth was still increasing at 20° C., and most of the curves at 25° show that the fungus grew more rapidly at this temperature than at 20°. At 30° C., however, the slope of all of the curves is greater than at 25°, indicating that a temperature of 30° is retarding in its effect. The curve for the cultures grown at pH 3.79 and at temperatures from 10° to 25° C., inclusive, has less slope than those for any other pH at these temperatures. This shows that *Sclerotinia fructicola* grew most rapidly on this particular medium at pH 3.79 at temperatures from 10° to 25° C. At 30° C., however, the slope of the curve for pH 3.79 is greater than it is for those of pH 6.42, 4.16, and 5.85. The rate of growth was greatest at 30° C. when the reaction was pH 5.85.

A better conception of the relative growth rates and of the effect of temperature on the growth rates at different hydrogen-ion concentrations is obtained if the data are analyzed on the basis of work. Growth, of course, represents a certain amount of work. There are two ways in which this can be considered: (a) the amounts of work (growth in this case) done in a given time, or (b) the times required, in each case, to do a given amount of work. Osterhout (17) and Smith (21) have pointed out that the latter method is more accurate and reliable.

The data presented in Appendices I, II, III, and IV were taken so that the time required to do a certain amount of work could be determined. In the case of *Sclerotinia fructicola* the number of hours required for the diameter of the mycelial disks to reach 30 millimeters is taken as a measure of the effici-

ency of the organism. A diameter of 50 millimeters was chosen for *Fomes annosus* because it is a more rapidly growing organism. These data are shown graphically in Figure 6.

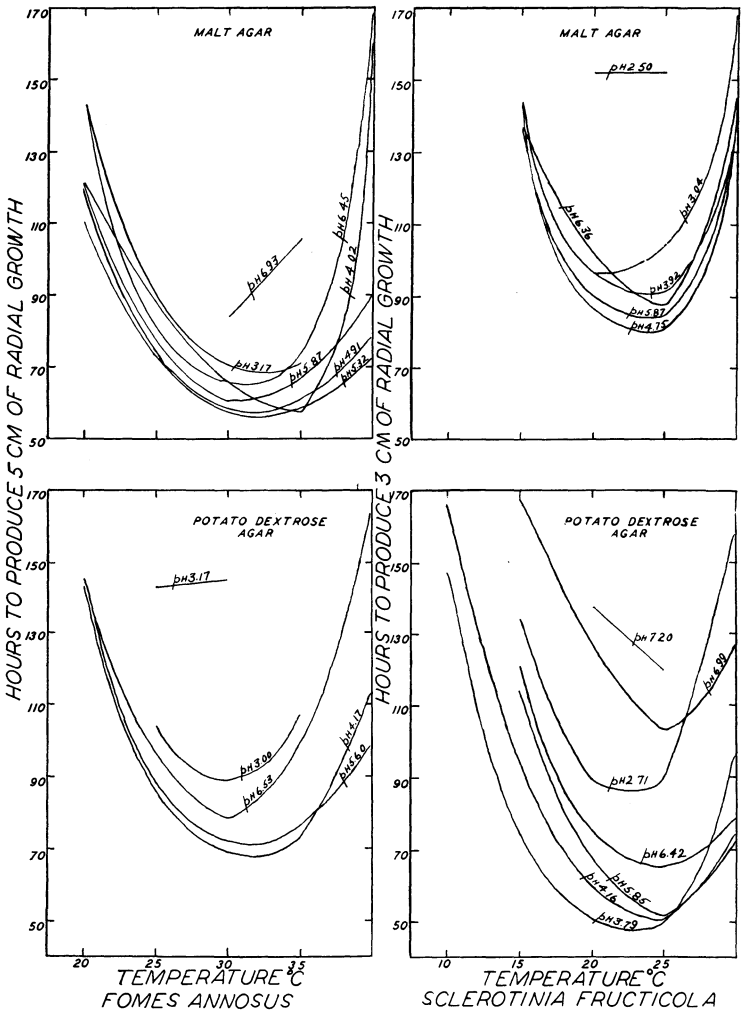

Fig. 6.—The effect of pH and temperature on the growth rate of *Fomes annosus* and *Sclerotinia fructicola*

The 5° C. temperature intervals are really too large to show accurately the exact temperature and pH at which growth progressed most rapidly and at which the organisms were the most efficient. To overcome this difficulty the number of hours required to produce the given amount of growth at the intervening temperatures has been interpolated from the original enlarged curves of Figure 6. While these values are a direct measure of the time required to

produce the same amount of growth and are inversely proportional to the efficiency of the organisms, it is a little clearer if the percentage of work accomplished per hour is calculated. This is done by taking the reciprocal of the number of hours required to do a given amount of work and multiplying it by 100. By comparing the percentages of work accomplished per hour, practically the same thing is done that Osterhout (17) suggested: ".... we can compare the reciprocals of the times required to bring the reaction to the same stage. If we merely wish to know the relative rates, as is usually the case in biology, it is not necessary to determine the velocity constants at all."

The term "efficiency factor" has been substituted for "percentage of work accomplished per hour". The efficiency factor is calculated by the following formula:

$$\text{Efficiency factor} = \frac{1}{\text{No. of hours required to do a given amount of work}} \times 100 = \text{Percentage of work accomplished per hour}$$

A direct means of comparing the growth rate of one culture with another, when the total amount of work or growth is the same, is furnished by the efficiency factors. For example, if the factors of two different cultures are 1.00 and 0.50, respectively, then the first culture did twice as much work per hour as the second culture; in other words, it grew twice as fast and a given amount of growth was produced in one-half of the time required by the second culture.

In Table 2 the number of hours required at each temperature for *Sclerotinia fructicola* to produce 30 millimeters of radial growth on potato dextrose agar at the different pH values and temperatures are given with the efficiency factors. Similar data have been calculated for the growth of this organism on malt agar and for *Fomes annosus* on both malt and potato dextrose agars. In the case of *Fomes annosus* the calculations were based on the number of hours required to produce 50 millimeters of radial growth. These data will be discussed but in order to conserve space are not presented in tabular form.

TABLE 2.—Number of Hours Required for *Sclerotinia fructicola* to Produce 30 Millimeters of Radial Growth, and the Efficiency Factors When Grown on Potato Dextrose Agar at Different Temperatures and pH Values

Temperature, °C.	Initial pH 2.71		Initial pH 3.79		Initial pH 4.16		Initial pH 5.85		Initial pH 6.42		Initial pH 6.99	
	Hours	E. F.	Hours	E. F.	Hours	E. F.	Hours	E. F.	Hours	E. F.	Hours	E. F.
10	.....	.....	147	0.69	166	0.60	.....	.....	.....	.....	.....	.....
11	.....	.....	127	0.78	154	0.64	.....	.....	.....	.....	.....	.....
12	.....	.....	111	0.90	143	0.69	.....	.....	.....	.....	.....	.....
13	.....	.....	96	1.04	131	0.76	.....	.....	.....	.....	.....	.....
14	.....	.....	83	1.20	120	0.83	.....	.....	.....	.....	.....	.....
15	134	0.75	72	1.38	108	0.92	114	0.87	121	0.82	168	0.59
16	123	0.81	66	1.51	95	1.05	105	0.95	110	0.90	160	0.62
17	114	0.87	61	1.63	84	1.19	96	1.04	101	0.99	151	0.66
18	105	0.95	56	1.78	74	1.35	86	1.16	91	1.09	143	0.69
19	97	1.03	53	1.88	67	1.49	76	1.31	82	1.21	135	0.74
20	89	1.12	51	1.96	59	1.69	68	1.47	75	1.33	127	0.78
21	86	1.16	50	2.00	55	1.81	61	1.63	69	1.44	120	0.83
22	84	1.19	49	2.04	52	1.92	55	1.81	66	1.51	113	0.88
23	85	1.17	49	2.04	50	2.00	52	1.92	64	1.56	108	0.92
24	86	1.16	49	2.04	50	2.00	50	2.00	63	1.58	104	0.96
25	89	1.12	50	2.00	50	2.00	51	1.96	64	1.56	102	0.98
26	95	1.05	54	1.85	54	1.85	53	1.88	66	1.51	103	0.97
27	104	0.96	62	1.61	58	1.72	57	1.75	69	1.44	104	0.96
28	119	0.84	74	1.35	63	1.58	62	1.61	72	1.38	109	0.91
29	136	0.73	83	1.20	68	1.47	66	1.51	75	1.33	118	0.84
30	158	0.63	96	1.04	74	1.35	72	1.38	78	1.28	127	0.78

A comparison of the efficiency factors for *Sclerotinia fructicola* given in Table 2, as well as an inspection of the curves in Figure 6, shows that the growth rate on potato dextrose agar was greater at pH 3.79 than at any other reaction tried from 10° to 24° C. At 25° and 26° C. the growth rate was the same at pH 3.79 and 4.16, but at 27°, 28°, 29°, and 30° growth progressed at a more rapid rate at pH 5.85 than at either 3.79 or 4.16. In other words, the optimum apparently shifted to a slightly more alkaline reaction at the higher temperatures. The growth rate was the fastest, as indicated by the efficiency factors, in the temperature range from 23° to 25° C. The efficiency factor is the largest at a reaction of pH 2.71 at 22° C., pH 3.79 at 22° to 24°, pH 4.16 at 23° to 25°, pH 5.85 at 24°, pH 6.42 at 24°, and pH 6.99 at 25°. On malt agar the situation is a little different in that the growth rate is the greatest at a higher reaction, pH 4.75, than was found to be the case on potato dextrose from 15° to 27° C.; at 28°, 29°, and 30° it shifts to a still higher reaction, pH 5.87. The temperatures at which growth occurred at the greatest rate on malt agar are from 22° to 24° C. and are about the same as those indicated above on potato dextrose agar. There is again a slight shift in the optimum temperature, from a lower to a higher value, as the hydrogen-ion concentration of the medium is decreased, the efficiency factor being the largest at 22° C. at pH 3.04 and at 24° at 6.36.

The efficiency factors, as well as the curves in Figure 6, for *Fomes annosus* grown on malt agar show that growth progressed most rapidly at pH 4.91 at 20° to 23° C., at 5.32 from 26° to 34°, at 4.02 from 34° to 35°, and at 5.32 from 36° to 40°. Growth occurred at the fastest rate in the case of each reaction tried in the temperature range extending from 30° to 35° C. On potato dextrose agar mycelial extension was most rapid at pH 4.17 at 20° to 37° C. and then shifted to 5.60 at from 38° to 40°. The range from 30° to 33° C. takes in the temperature at each pH value where mycelial extension is the most rapid.

Sclerotinia fructicola grew much more efficiently on potato dextrose agar than on malt agar. The largest efficiency factor on potato dextrose agar was 2.04 at initial pH 3.79 and at temperatures from 22° to 24° C.; whereas on malt agar the largest factor was 1.26 at pH 4.75 at 23° and 24°. At the temperature and pH, then, where mycelial extension was the most rapid this organism accomplished 0.78 per cent more of the work per hour on potato dextrose than on malt agar necessary to produce 30 millimeters of radial growth. *Fomes annosus*, on the other hand, was the most efficient on malt agar. The largest efficiency factor for this organism was 1.81 at pH 4.02 and at 35° C.; whereas on potato dextrose agar the largest factor was 1.40 at pH 4.17 and at 31° and 33°.

It is evident from the above considerations of the efficiency factors and from a critical examination of the curves in Figure 6 that the hydrogen-ion concentration of the media at which the mycelial disks of *Sclerotinia fructicola* and *Fomes annosus* enlarge the most rapidly (at least up to 30 and 50 millimeters, respectively) is not constant when the temperature is varied. There is a shift to a less acid reaction at the higher temperatures. The temperatures which are the most favorable for mycelial extension are roughly the same at all pH values, but there is a tendency in some cases for the optimum temperature to be a degree or two lower on the acid side of the range than on the less acid side. These facts would seem to be evidence supporting the statement made earlier in the discussion that there is a tendency for the toxicity of the hydrogen ion to be greatest at the upper temperature limits and, conversely, for the hydroxyl ion to be less toxic at the upper than at the lower limits.

Effect of temperature on the optimum pH.—If the optimum pH for the growth of a fungus is defined as that reaction of the medium at which mycelial extension progresses at the most rapid rate during a specified time, the preceding discussion shows that the optimum reaction varies with the temperature and constitution of the media. On potato dextrose agar the optimum pH for *Sclerotinia fructicola* was near 3.79 at temperatures from 10° to 24° C., 3.79 to 4.16 from 25° to 26°, and near 5.85 from 27° to 30°. In the case of *Fomes annosus* on malt agar the optimum pH was near 4.91 at temperatures from 20° to 23° C. and 5.32 from 26° to 40°; whereas on potato dextrose agar the optimum was near 4.17 from 20° to 37° and 5.60 from 38° to 40°. The optimum pH for growth of these two organisms depends on the medium and on the temperature, becoming higher as the temperature increases.

Temperature coefficients.—A temperature coefficient may be defined as the ratio of the rate of a given process at any given temperature to the rate at another temperature, at a fixed interval below the first temperature. The temperature interval is usually taken as 10° C. and the coefficient for a 10° rise in temperature is indicated by the symbol Q_{10}. Van't Hoff showed that for most chemical reactions the temperature coefficient is from 2 to 3. Temperature coefficients have been worked out for a good many physiological processes in biology.

The coefficients are given in Table 3 for the rate of mycelial extension for *Sclerotinia fructicola* grown on potato dextrose agar and for *Fomes annosus* on malt agar of different hydrogen-ion concentrations for 10° intervals, calculated in part from the data in Table 2. These values are seen to vary greatly, with the larger coefficients occurring for the lower and the smaller ones for the higher portion of the temperature range. Undoubtedly, if data were available for still lower temperatures the coefficients would be much

TABLE 3.—Temperature Coefficients for Mycelial Extension of *Sclerotinia fructicola* and *Fomes annosus* at Different Hydrogen-Ion Concentrations

Organism and medium	Temperature range, °C.	Temperature coefficients at initial					
		pH 2.71	pH 3.79	pH 4.16	pH 5.85	pH 6.42	pH 6.99
S. fructicola Potato dextrose agar	10-20	.....	2.89	2.82	.....	.....	.....
	11-21	.....	2.54	2.80	.....	.....	.....
	12-22	.....	2.24	2.75	.....	.....	.....
	13-23	.....	1.96	2.62	.....	.....	.....
	14-24	.....	1.69	2.40	.....	.....	.....
	15-25	1.50	1.44	2.16	2.22	1.88	1.65
	16-26	1.29	1.22	1.75	1.98	1.68	1.55
	17-27	1.10	0.98	1.45	1.68	1.46	1.45
	18-28	0.88	0.75	1.17	1.39	1.26	1.31
	19-29	0.71	0.64	0.98	1.15	1.09	1.14
	20-30	0.51	0.52	0.80	0.94	0.96	1.00
F. annosus Malt agar		pH 3.17	pH 4.02	pH 4.91	pH 5.32	pH 5.87	pH 6.45
	20-30	2.00	2.02	1.84	2.02	2.03	2.12
	21-31	1.84	1.98	1.68	1.88	1.91	1.94
	22-32	1.68	1.88	1.53	1.68	1.75	1.78
	23-33	1.53	1.80	1.42	1.51	1.59	1.55
	24-34	1.35	1.63	1.28	1.35	1.43	1.33
	25-35	1.25	1.36	1.11	1.22	1.26	1.08
	26-36	.....	1.23	1.08	1.11	1.11	0.89
	27-37	.....	0.81	0.97	1.00	0.95	0.71
	28-38	.....	0.63	0.87	0.92	0.81	0.56
	29-39	.....	0.50	0.82	0.85	0.72	0.47
	30-40	.....	0.46	0.77	0.80	0.65	0.39

greater. They are, however, in the order to be expected from this type of growth data. At a very low temperature, near the absolute minimum for growth, Q_{10} would be very large and at increasingly higher temperatures, above the optimum and approaching the maximum, very small; in fact, it would approach zero. The results are similar to those reported by Fawcett (9) in this respect. The largest coefficient he found was 30 (range 8° to 18°) for the first 24-hour period after inoculation with *Phytophthora terrestria* and the smallest was 0.01 (range 26° to 36°) for the fourth 24-hour period. Smith (21) reports some very high coefficients for the killing of *Botrytis* spores by heat. He determined the length of exposure to temperatures of 31° and 37° C. necessary to kill 50 per cent of the spores. He states that, if the rate had progressed on up to 41° C. as fast as it did from 31° to 37°, Q_{10} would have been 690. The coefficient for the range 37° to 47° he reported as 120.

When growth rates are compared at different temperatures, especially near the temperature limits for growth, Q_{10} can hardly be expected to be near 2 or 3, as would be the case if the process were a simple chemical one involving a single reaction. Growth is really a "constellation" of activities and the magnitude of the temperature effect on the whole may be determined, more or less, by its effect on any one activity; in other words, the one may become a limiting factor. Cohen (4), Fawcett (9), and Smith (21) have advanced such viewpoints, and Snyder (22) has attempted to explain the meaning of some of the variations in magnitude of temperature coefficients.

DISCUSSION

The effect of temperature and of the hydrogen-ion concentration of the medium on spore germination and the rate of growth of fungous mycelium was not constant for the two organisms used. Either of these two environmental factors was influenced in effect by the intensity of the other factor. The pH range permitting the germination of spores or the growth of mycelium was dependent on the temperature. At favorable temperatures the range reached a maximum for the particular organism; whereas at unfavorable temperatures it was reduced. Even the optimum pH for mycelial growth was not constant throughout the temperature range but shifted to a less acid reaction with increased temperature.

Other environmental factors probably are interrelated with respect to their effect on the activities of fungi in the same manner as pH and temperature are. The effect of each individual factor may be influenced by the intensity of any or all of the others in the environmental complex. A given factor, for instance, may be slightly unfavorable and growth may not occur if one or more other factors are unfavorable; whereas growth may progress fairly rapidly if the other conditions are favorable.

SUMMARY

1. The effect of hydrogen-ion concentration on the germination of conidia of *Sclerotinia fructicola* after 24 hours was found intimately related to the temperature with respect to the limits of pH for germination. The widest reaction range occurred at 21° C. and was from pH 1.4 to 7.2. Temperature limits were influenced, likewise, by the reaction.

2. The optimum reaction for spore germination was not influenced materially by the temperature, being near pH 2.4 at all temperatures tried, except at 33° C. At this temperature germination was best at pH 5.6. Hydrogen-ion concentration, on the other hand, did influence the optimum temperature.

3. Double maxima germination curves were obtained at every temperature tried, the minimum being at pH 6.0.

4. Both *Sclerotinia fructicola* and *Fomes annosus* produced large amounts of acid when grown on potato dextrose agar and on malt agar. The final pH of the phosphate buffered media on the more alkaline side of the range was lower by over two pH units in some instances than the initial pH.

5. The growth curves for both organisms, with a few exceptions, were straight lines during the course of the experiments when the lag phase was ignored.

6. The hydrogen ion was found much less toxic than the hydroxyl ion. There is an indication that the hydrogen ion was more toxic at the upper than at the lower temperature limits for growth; whereas the hydroxyl ion apparently tended to be more toxic at the lower temperature limits.

7. The pH limits for growth were influenced by the temperature, the range being the widest at the most favorable temperatures. Temperature limits were influenced, likewise, by the reaction of the media.

8. The efficiency of the two fungi in producing a given amount of radial growth was dependent on the media, the hydrogen-ion concentration, and the temperature. *Sclerotinia fructicola* was more efficient on potato dextrose agar and *Fomes annosus* on malt agar. In general, the reaction of the media most favorable for mycelial extension was not constant when the temperature was varied. There was a shift to a less acid reaction at the higher temperatures.

9. At the higher temperatures the optimum pH for mycelial extension was higher than at the lower temperatures. The optimum pH for growth was higher than for spore germination with *Sclerotinia fructicola.*

10. Temperature coefficients were calculated for the rate of growth of *Sclerotinia fructicola* on potato dextrose agar and for *Fomes annosus* on malt agar at the different hydrogen-ion concentrations. These were found to vary greatly throughout the temperature range, being the largest for the lower portion and the smallest for the higher portion of the range.

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LITERATURE CITED

1. Bateman, E. 1933. The effect of concentration on the toxicity of chemicals to living organisms. U. S. Dept. Agr. Tech. Bull. 346, 1-53.
2. Brooks, C. 1906. Temperature and toxic action. Bot. Gaz. **42**: 359-375.
3. Clark, W. M. and H. A. Lubs. 1917. The colorimetric determination of hydrogen ion concentrations and its application in bacteriology. Jour. Bact. **2**: 1-34, 109-136, 191-236.
4. Cohen, B. 1922. Disinfection studies: The effect of temperature and hydrogen ion concentration upon the viability of *B. coli* and *B. typhosum* in water. Jour. Bact. **7**: 183-230.
5. Cooley, J. S. 1914. A study of the physiological relations of *Sclerotinia cinerea* (Bon.) Schröter. Ann. Missouri Bot. Gard. **1**: 291-326.
6. Cummins, J. E. 1926. The effect of hydrogen ion concentration on the growth of *Fomes annosus*. Univ. of Wisc. Thesis.
7. Duggar, B. M. 1901. Physiological studies with reference to the germination of certain fungous spores. Bot. Gaz. **31**: 38-66.
8. Dunn, M. S. 1926. Effects of certain acids and their sodium salts upon the growth of *Sclerotinia cinerea*. Amer. Jour. Bot. **13**: 48-58.
9. Fawcett, H. S. 1921. The temperature relations of growth in certain parasitic fungi. Univ. Calif. Publ. in Agr. Sci. **4**: 183-232.
10. Hopkins, E. F. 1922. Hydrogen ion concentration in its relation to wheat scab. Amer. Jour. Bot. **9**: 159-179.
11. Hursch, C. R. 1922. The relation of temperature and hydrogen ion concentration to urediniospore germination of biologic forms of stem rust of wheat. Phytopath. **12**: 353-361.
12. Itano, A. and J. Neil. 1919. Influence of temperature and hydrogen ion concentration upon the spore cycle of *Bacillus subtilis*. Jour. Gen. Physiol. **1**: 421-428.
13. Johnson, H. W. 1923. Relationships between hydrogen ion, hydroxyl ion and salt concentrations and the growth of seven soil molds. Iowa Agr. Exp. Sta. Res. Bull. 76, 305-344.
14. Klotz, L. J. 1923. Studies in the physiology of the fungi. XVI. Some aspects of nitrogen metabolism in fungi. Ann. Missouri Bot. Gard. **10**: 299-368.
15. Lindfors, T. 1924. Einige Kulturversuche mit Fusarium-Arten in Nährlösungen von verschiedener Wasserstoffionenkonzentration. Bot. Not. (1924), pp. 161-171.
16. MacInnes, J. 1922. The growth of the wheat scab organism in relation to hydrogen ion concentration. Phytopath. **12**: 290-294.
17. Osterhout, W. J. V. 1918. Note on measuring the relative rates of life processes. Science, N. S., **48**: 172.
18. Robbins, W. J. 1934. Isoelectric points for the mycelium of fungi. Jour. Gen. Physiol. **6**: 259-271.
19. Scott, I. T. 1924. The influence of hydrogen ion concentration on the growth of *Fusarium lycopersici* and on tomato wilt. Missouri Agr. Exp. Sta. Res. Bull. 64, 1-32.

20. ————. 1929. Hydrogen ion equilibrium of mycelial mats of *Fusarium lycopersici* in salt solutions and its relation to growth and toxicity. Amer. Jour. Bot. **16**: 631-643.

21. Smith, J. H. 1923. The killing of *Botrytis cinerea* by heat, with a note on the determination of temperature coefficients. Ann. Appl. Biol. **10**: 335-347.

22. Snyder, C. D. 1911. On the meaning of variations in the magnitude of temperature coefficients of physiological processes. Amer. Jour. Physiol. **28**: 167-175.

23. Tilford, P. E. 1935. The use of the quinhydrone and antimony electrodes for determining the pH of solid culture media. Phytopath. **25**: 362-367.

24. Walbum, L. E. 1920. Über die Wasserstoffionenkonzentration einiger Standardlösungen bei verschiedenen Temperaturen. Biochem. Zeit. **107**: 219-228.

25. Webb, R. W. 1919. Studies in the physiology of the fungi. X. Germination of the spores of certain fungi in relation to hydrogen ion concentration. Ann. Missouri Bot. Gard. **6**: 201-222.

26. ————. 1921. Studies in the physiology of the fungi. XV. Germination of the spores of certain fungi in relation to the hydrogen ion concentration. Ann. Missouri Bot. Gard. **8**: 283-341.

27. ———— and H. Fellows. 1926. The growth of *Ophiobolus graminis* Sacc. in relation to hydrogen ion concentration. Jour. Agr. Res. **33**: 845-872.

28. Wehmer, C. 1891. Entstehung und physiologische Bedeutung der Oxalsäure im Stoffwechsel einiger Pilze. Bot. Zeit. **49**: 233-246, 249-257, 271-280, 289-298, 305-313, 321-332, 337-346, 353-363, 369-374, 385-396, 401-407, 417-428, 433-439, 449-456, 465-478, 511-518, 531-539, 547-554, 563-569, 579-584, 596-602, 611-620, 630-638.

29. Weimer, J. L. and L. L. Harter. 1923. Hydrogen ion changes induced by species of Rhizopus and by *Botrytis cinerea*. Jour. Agr. Res. **25**: 155-164.

30. Wolpert, F. S. 1924. Studies in the physiology of the fungi. XVII. The growth of certain wood-destroying fungi in relation to the H-ion concentration of the media. Ann. Missouri Bot. Gard. **11**: 43-97.

31. Young, H. C. and C. W. Bennett. 1922. Growth of some parasitic fungi in synthetic culture media. Amer. Jour. Bot. **9**: 459-469.

APPENDIX TABLE I.—The Effect of H-ion Concentration and Temperature on the Rate of Radial Growth of *Sclerotinia fructicola* on Malt Agar

Initial pH	Temperature, °C	Millimeters of radial growth after 48 hours	56 hours	72 hours	80 hours	96 hours	104 hours	120 hours	144 hours	152 hours	168 hours
2.15	10	.....	.....	.....	.....	.....	.....	.....	5.0	.....	5.0
	15	.....	.....	4.0	5.5	7.5	8.0	10.0	11.5	12.0	13.5
	20	3.0	6.0	7.0	8.0	11.0	11.0	14.0	16.5	17.5	20.5
	25	5.5	7.0	10.0	10.0	12.5	12.5	15.5	21.0	21.0	24.5
	30	.....	4.0	5.0	.....	5.0	5.0	.....	5.0	.....	5.0
	35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.50	10	.....	.....	1.5	.....	5.0	.....	5.5	6.0	.....	7.5
	15	3.0	5.5	8.0	9.0	11.0	12.0	15.0	19.0	19.0	21.0
	20	9.0	11.5	14.5	16.5	20.0	20.5	24.0	29.0	30.0	.....
	25	9.5	10.5	15.0	16.5	18.5	19.0	23.0	29.0	30.0	.....
	30	6.0	6.0	9.5	11.0	11.5	13.0	13.5	16.5	18.0	20.0
	35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3.04	10	.....	.....	1.0	.....	3.5	.....	5.5	6.5	.....	9.0
	15	5.0	10.0	12.0	15.0	20.0	20.0	25.0	30.0	.....	.....
	20	13.0	17.5	23.0	25.0	30.0	.....	.....	.....	.....	.....
	25	15.0	18.0	22.0	23.5	28.0	30.0	.....	.....	.....	.....
	30	11.0	14.0	15.0	16.0	18.5	19.5	22.0	26.0	28.5	30.5
	35	8.0	.....	11.0	.....	13.0	.....	13.5	13.5	.....	13.5
3.92	10	.....	.....	3.0	.....	7.0	.....	8.5	.....	.....	10.5
	15	7.0	10.0	13.0	15.0	19.0	21.0	24.5	30.0	.....	.....
	20	14.0	17.0	22.0	24.5	30.0	.....	.....	.....	.....	.....
	25	16.5	20.0	25.0	27.5	31.5	.....	.....	.....	.....	.....
	30	13.0	15.0	17.0	19.0	22.0	22.5	27.0	32.5*	.....	.....
	35	6.0	.....	10.0	.....	10.0	.....	11.0	.....	11.0	11.0
4.75	10	.....	.....	4.0	.....	7.5	.....	8.5	10.5	.....	13.5
	15	9.0	10.0	15.0	17.0	20.0	22.5	25.5	30.5	.....	.....
	20	17.0	20.5	25.0	27.5	33.5	.....	.....	.....	.....	.....
	25	19.0	21.0	26.0	30.0	.....	.....	.....	.....	.....	.....
	30	11.0	13.0	15.0	17.5	19.5	20.0	23.0	25.0	30.0	.....
	35	7.0	.....	10.5	.....	14.0	.....	15.0	16.0	.....	18.0

Initial pH	Temperature, °C	Millimeters of radial growth after 41 hours	49 hours	65 hours	90 hours	113 hours	121 hours	137 hours	145 hours	161 hours
5.87	10	.....	.....	.....	5.0	.....	.....	.....	.....	11.0
	15	4.0	.....	6.5	15.0	22.5	24.5	30.0	.....	.....
	20	12.0	15.0	20.0	30.0	.....	.....	.....	.....	.....
	25	13.5	17.0	24.0	32.0	.....	.....	.....	.....	.....
	30	11.0	12.0	16.0	20.5	25.0	25.5	30.0	.....	.....
	35	8.0	.....	10.5	15.0	17.5	.....	.....	.....	21.5
6.36	10	.....	.....	.....	2.0	.....	.....	.....	.....	9.0
	15	6.0	.....	11.0	17.0	24.0	26.0	30.0	.....	.....
	20	12.0	15.0	20.5	28.5	37.0	.....	.....	.....	.....
	25	15.0	17.5*	23.0*	31.5*	.....	.....	.....	.....	.....
	30	6.0	6.0	10.0	14.0	22.0	23.0	27.0	30.0	.....
	35	7.5	.....	12.0	16.0	18.0	.....	.....	.....	20.0
6.80	10	.....	.....	.....	.....	.....	.....	.....	.....	4.5
	15	2.5	.....	5.0	7.0	10.0	11.0	12.0	14.0	15.0
	20	6.5	6.5	8.0	10.0	13.0	14.0	17.5	18.5	24.0
	25	6.0*	7.0*	8.0*	10.0*	17.0*	20.0*	21.0*	24.0*	30.0*
	30	5.0	7.0	8.0	16.0	.....	.....	18.0	18.0	20.0
	35	.....	.....	.....	.....	.....	.....	.....	.....	.....
7.25	10	.....	.....	.....	.....	.....	.....	.....	.....	2.0
	15	.....	.....	5.0	8.0	11.0	12.0	12.5	15.0	15.0
	20	6.0	8.0	10.0	12.0	13.5	14.0	16.5	16.5	18.0
	25	5.0	7.5	8.0	11.0	14.0	14.0	16.0	17.0	18.0
	30	2.0	.....	4.0	5.0	6.5*	6.5*	6.5*	6.5*	7.0*
	35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Data are from one plate only. The other plate was discarded because of contamination, breakage, or failure of the fungus to grow.

APPENDIX TABLE II.—The Effect of H-ion Concentration and Temperature on the Rate of Radial Growth of *Sclerotinia fructicola* on Potato Dextrose Agar

Initial pH	Temp., °C.	Millimeters of radial growth after									
		40 hours	64 hours	88 hours	112 hours	136 hours	160 hours				
2.20	10	0.0	2.0	4.0	6.0	9.0	10.5				
	15	3.0	5.0	8.0	11.5	14.0	17.0				
	20	4.0	8.0	13.0	15.5	18.0	21.5				
	25	5.0	8.5	11.5	15.5	17.5	20.0				
	30	2.5	6.0	9.0	11.5	14.0	18.0				
		41 hours	65 hours	90 hours	113 hours	137 hours	161 hours				
2.71	10	.....	8.5	12.0	15.0	18.5	22.0				
	15	9.0	14.0	20.0	25.5	31.0	.....				
	20	14.0	21.5	30.0	.....	.....	.....				
	25	12.5	20.5	30.0	.....	.....	.....				
	30	10.0	13.0	23.5	24.0	27.0	31.0				
		25 hours	32 hours	49 hours	56 hours	72 hours	96 hours	120 hours	168 hours		
3.79	10	.....	.....	.....	.....	10.5*	18.5*	23.5*	35.0*		
	15	.....	.....	17.5	20.0	30.0	.....	.....	.....		
	20	10.0	15.5	28.0	34.0	.....	.....	.....	.....		
	25	10.0	16.5	30.0	.....	.....	.....	.....	.....		
	30	.....	.....	16.0	19.5	23.0	30.0	.....	.....		
		24 hours	30 hours	45 hours	54 hours	70 hours	79 hours	94 hours	118 hours	142 hours	168 hours
4.16	10	.....	.....	.....	.....	8.5	.....	14.5	21.0	25.0	30.0
	15	.....	.....	7.5	10.0	17.0	19.5	25.0	33.0	.....	.....
	20	8.5	13.0	23.0	27.0	35.0	.....	.....	.....	.....	.....
	25	10.0	16.0	26.0	32.5	.....	.....	.....	.....	.....	.....
	30	5.0	.....	14.5	19.5	29.0	32.0	.....	.....	.....	.....
		41 hours	48 hours	64 hours	72 hours	89 hours	96 hours	113 hours	137 hours	161 hours	168 hours
5.85	10	.....	.....	.....	.....	7.0	.....	10.0	13.5	16.5	19.5
	15	8.0	11.0	15.5	18.0	23.5	25.5	31.0	.....	.....	.....
	20	16.5	20.0	27.0	32.0	.....	.....	.....	.....	.....	.....
	25	22.0	28.0	39.0	.....	.....	.....	.....	.....	.....	.....
	30	13.5	18.0	25.0	29.5	.....	.....	.....	.....	.....	.....
6.42	10	.....	.....	.....	.....	7.0	.....	10.0	13.0	16.0	18.5
	15	8.0	10.0	14.5	16.5	22.0	23.5	28.0	35.0	.....	.....
	20	12.0	16.0	23.0	27.5	35.0	.....	.....	.....	.....	.....
	25	17.5*	22.0*	30.0*	.....	.....	.....	.....	.....	.....	.....
	30	14.0	17.5	24.5	28.0	35.0	.....	.....	.....	.....	.....
6.99	10	.....	.....	.....	.....	5.0	.....	7.5	10.5	11.5	14.0
	15	5.0	6.0	9.0	10.5	13.0	14.0	16.5	21.5	27.0	29.0
	20	6.0	8.0	11.5	13.0	16.0	18.5	22.5	30.5	.....	.....
	25	7.5	11.0	16.0	19.0	27.5	29.0	34.0	.....	.....	.....
	30	4.5*	6.0*	8.0*	9.0*	16.5*	19.0*	25.0*	33.0*	.....	.....
		40 hours	88 hours	120 hours	138 hours	162 hours					
7.20	10	0.0	0.0	0.0	0.0	0.0					
	15	.....	10.0	.....	.....	20.0					
	20	7.0	16.5	25.0	30.0	.....					
	25	8.0	20.5	32.5	.....	.....					
	30	.....	13.5*	20.0*	25.0*	28.0*					
7.94	20	.....	12.0	15.0	18.0	22.0					
	25	.....	11.5*	18.0*	18.0*	18.0*					
	30	.....	7.5	9.5	.....	10.0					

*Data are from one plate only. The other plate was discarded because of contamination, breakage, or failure of the fungus to grow.

APPENDIX TABLE III.—The Effect of H-ion Concentration and Temperature on the Rate of Radial Growth of *Fomes annosus* on Malt Agar

Initial pH	Temp., °C.	Millimeters of radial growth after							
		46 hours	54 hours	71 hours	78 hours	93 hours	121 hours	142 hours	168 hours
2.60	15	.....	1.0	5.0	6.5	7.5	10.0	11.5	14.0
	20	.....	5.5	10.0	13.7	17.0	22.0	27.5	36.5
	25	.....	6.0*	13.0*	15.0*	17.0*	23.0*	30.0*	35.0*
	30	.....	6.0*	10.0*	12.0*	15.0*	17.0*	17.0*	20.0*
	35	.....	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	40	.....	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3.17	15	0.0	1.0	5.0	6.0	7.5	10.0	12.0	15.0
	20	7.0	13.5	17.5	21.0	27.0	37.0	50.0	.....
	25	19.0	24.5	37.0	41.5	52.5	.....	.....	.....
	30	25.0	34.5	50.0	.....	.....	.....	.....	.....
	35	30.0	33.0	49.0	.....	.....	.....	.....	.....
	40	0.0	3.0	5.0	6.0	9.0	12.0	12.0	12.0
4.02	15	0.0	3.0	6.5	10.0	14.0	20.0	25.0	32.0
	20	9.0	17.0	25.0	30.0	36.0	50.0	.....	.....
	25	16.0	22.0	37.0	42.0	55.0	.....	.....	.....
	30	25.0*	35.0*	51.0*	.....	.....	.....	.....	.....
	35	38.0	44.5	66.0	.....	.....	.....	.....	.....
	40	18.0*	18.0*	22.0*	24.0*	30.0*	37.5*	43.0*	52.0*
4.91	15	0.0	5.5	9.7	13.0	16.5	22.0	26.0	36.5
	20	7.5	15.0	23.0	29.0	39.0	57.0	.....	.....
	25	26.5	34.5	50.0	.....	.....	.....	.....	.....
	30	33.5	45.0	62.0	.....	.....	.....	.....	.....
	35	38.0	43.5	64.0	.....	.....	.....	.....	.....
	40	23.0	30.0*	46.0*	50.0*	.....	.....	.....	.....
		48 hours	54 hours	72 hours	78 hours	96 hours	119 hours	143 hours	168 hours
5.87	15	0.0	0.0	4.0	.....	10.0	.....	12.0	12.0
	20	16.0	19.0	26.0	29.0	37.0	50.0	.....	.....
	25	29.0	32.5	45.5	53.0	.....	.....	.....	.....
	30	39.5	42.5	66.0	.....	.....	.....	.....	.....
	35	31.5	32.7	40.0	41.0	46.0	.....	.....	.....
	40	22.0	25.0	40.0	43.0	55.0	.....	.....	.....
6.45	15	0.0	0.0	2.0	.....	.....	8.0	10.0	15.0
	20	9.5	14.0	18.5	23.0	30.0	37.5	50.0	.....
	25	26.0	30.5	45.0	47.5	65.0	.....	.....	.....
	30	35.0	40.0	54.5	.....	.....	.....	.....	.....
	35	31.0	36.0	50.0	.....	.....	.....	.....	.....
	40	11.0*	13.0*	20.0*	.....	28.0*	33.0*	41.0*	50.0*
6.93	15	0.0	0.0	2.0	.....	.....	.....	3.0	5.0
	20	8.5	10.5	15.5	16.5	22.0	.....	27.5	33.5
	25	17.7	21.0	31.5	32.5	43.5	47.5	.....	.....
	30	24.5	28.5	41.5	43.0	58.5	.....	.....	.....
	35	17.5	20.0	.....	36.0	47.0	51.0	.....	.....
	40	3.0	4.0	6.5	7.0	8.0	9.0	9.0	9.0
7.54	15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	20	0.0	0.0	0.0	0.0	2.0	5.5	10.5	15.0
	25	0.0	0.0	3.0	3.0	12.5	19.5	30.5	40.7
	30	2.5	5.5	12.5	13.0	20.5	32.0	45.0	60.0
	35	6.0	10.0	15.0	20.0	30.0	40.0	50.0	.....
	40	0.0	0.0	0.0	0.0	0.0	0.0	0.0	.....

*Data are from one plate only. The other plate was discarded because of contamination, breakage, or failure of the fungus to grow.

APPENDIX TABLE IV.—The Effect of H-ion Concentration and Temperature on the Rate of Radial Growth of *Fomes annosus* on Potato Dextrose Agar

Initial pH	Temp., °C.	Millimeters of radial growth after							
		48 hours	72 hours	79 hours	101 hours	121 hours	145 hours	169 hours	
2.63	15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	20	0.0	2.5	3.5	5.5	13.0	19.5	26.0	
	25	10.0*	21.0*	23.5*	34.0*	41.0*	51.0*	.....	
	30	7.0	17.5	20.0	30.0	38.0	50.0	.....	
	35	0.0	0.0	2.0	4.0	6.0	8.0	10.0	
	40	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
3.00	15	0.0	0.0	0.0	0.0	2.0*	3.0*	5.0*	
	20	0.0	0.0	4.0	8.0	13.5	23.0	32.5	
	25	11.0	25.0	31.5	45.5	63.0	.....	.....	
	30	16.0	36.5	41.0	62.0	.....	.....	.....	
	35	6.0	21.7	27.0	44.0	63.0	.....	.....	
	40	0.0	0.0	0.0	3.0	4.0	5.0	6.0	
4.17	15	0.0	0.0	2.0	5.0	10.0	16.0	22.0	
	20	4.5	13.0	16.0	26.5	42.0	51.0	.....	
	25	17.0	38.0	45.0	65.0	.....	.....	.....	
	30	16.5	40.0	47.5	72.5	.....	.....	.....	
	35	22.0	50.0	.....	.....	.....	.....	.....	
	40	7.5	27.0	32.0	44.5	54.5	.....	.....	
5.60	15	0.0	3.0	5.0	10.0	15.0	23.0	30.0	
	20	4.0*	12.0*	17.0*	28.0*	40.0*	50.0*	.....	
	25	14.0	38.0	44.0	62.5	.....	.....	.....	
	30	24.0	48.0	55.0	.....	.....	.....	.....	
	35	18.5	44.0	54.0	.....	.....	.....	.....	
	40	10.0	34.0	39.0	51.0	.....	.....	.....	
		47 hours	71 hours	78 hours	95 hours	102 hours	119 hours	143 hours	167 hours
6.53	15	0.0	4.0*	6.0*	14.0*	15.0*	23.0*	30.0*	36.0*
	20	5.0	18.0	25.0	30.0	32.0	36.0	50.0	.....
	25	10.0	28.0	34.0	46.0	52.0	.....	.....	.....
	30	20.0	43.5	50.0	.....	.....	.....	.....	.....
	35	17.0	29.0	33.0	41.0	45.5	55.0	.....	.....
	40	3.0	16.0	.....	20.0	25.0	32.0	40.0	53.0
7.13	15			Both cultures contaminated					
	20	3.0	7.5	8.5	13.5	14.5	19.0	28.0	39.0
	25	7.5	15.0	17.5	27.0	30.0	43.5	58.0	.....
	30	12.0	20.0	24.0	35.0	41.0	51.0	.....	.....
	35	0.0	5.0*	7.0*	12.0*	14.0*	20.0*	31.0*	40.0*
	40	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Data are from one plate only. The other plate was discarded because of contamination, breakage, or failure of the fungus to grow.

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